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RESEARCH ARTICLE

Nutritionally improved pasta with Arthrospira platensis: effect of cooking on antioxidant capacity and pigments content

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ABSTRACT

The functionalizing of staple and economic foods, which means adding health-promoting substances, has been visualized as a solution to reduce the concerning increase in diet-related diseases caused by bad-eating patterns. Microalgae represent an innovative way to solve this problem. *Arthrospira platensis*, microalgae nutritionally rich in bioactive compounds, has been used to develop hard wheat pasta (semolina) to improve its nutritional value and provide antioxidant properties, but there is scarce information about the effect of adding *A. platensis* in a soft wheat pasta with egg on these parameters. Given that, this work aimed to assess the effect of adding *A. platensis* at 1, 5, and 10% in a soft wheat pasta added with egg on the nutritional value. Besides, the total phenolic content (TPC), antioxidant capacity (by FRAP and ABTS), and the spectrophotometric estimation of chlorophylls a + b as well as total carotenoids content were determined after and before cooking. The results showed that adding *A. platensis* at 5% was enough to increase raw pasta's nutritional value, especially protein by 19.27%, TPC by 3.88%, antioxidant capacity by 48.54%, and 66.09% for ABTS and FRAP respectively, as well as chlorophyll a + b (5.89 mg/100g) and total carotenoids (1.31 mg/100g). After the cooking process losses of 7.40, 16.81 and 0.51% were evidenced for TPC, ABTS and FRAP assays, however, remained 10.63, 62.37 and 70.65% higher than the cooked control. Furthermore, increases of 92.19 and 54.96% for chlorophyll a+b and total carotenoids were evidenced. The addition of *A. platensis* to pasta represents a way to improve the nutritional value regarding protein content, increase antioxidant capacity, and the content of chlorophylls and carotenoids, without statistically significant modifications in the caloric content.

Keywords: Antioxidant; *Arthrospira platensis;* Carotenoids; Chlorophylls; Functional Food; Pasta

INTRODUCTION

Wheat flour pasta represents a staple food product in many countries mainly to its food characteristics, like convenience, palatability, nutritional quality, relative non-perishable capacity, and affordable cost (Kaur et al., 2017; El-Hameed et al., 2018). Commonly hard wheat (*Triticum turgidum* L. var. Durum) is used to produce pasta, but it is 20.25% more expensive than soft wheat (*Triticum aestivum*) (Zavalishina et al., 2021). Frequently pasta with higher density, firmness, and enhanced elastic properties is preferred, and soft wheat can confer these (Nilusha *et al.*, 2019).

Pasta is a good source of carbohydrates but lacks enough nutrients like vitamins, minerals, and phytochemicals (Kaur *et al.*, 2017). This nutrimental deterioration occurs in wheat grain milling during the elaboration of flour, where a considerable decrease in vitamins, minerals, fiber, fat, protein, and carbohydrate content modification occurs in the function of the grinding fineness (Rosado *et al.*, 1999).

Due to the abrupt increase in health problems related to unhealthy dietary patterns, a market niche is focused on the development of foods that can prevent diseases, recurring to the use of bioactive substances as antioxidants from vegetables and edible plants (Boroski *et al.*, 2011). Foods with such properties are termed functional foods and are defined as natural-occurring of processed foods with health-promoting or disease-preventing properties beyond their traditional nutritional value (Ememe and Ememe, 2017).

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Since pasta is a globally consumed food, popular, and has a low price and long shelf life, it is visualized as a vehicle for adding nutrients. This consideration is also supported by the Food and Drug Administration (FDA) and the World Health Organization (WHO) (Nilusha *et al.*, 2019). Targeting such staple foods of wide acceptance or frequent consumption as vehicles makes it possible to distribute bioactive compounds to the population to promote overall health (Caporgno and Mathys, 2018).

Microalgae as food is a poorly explored natural source for a healthy diet since many are rich in carbohydrates, proteins, lipids, and valuable compounds of nutritional interest (Sathasivam *et al.*, 2019). These aspects make microalgae a potential food ingredient of high nutritional value and health benefits (Batista *et al.*, 2019). *Arthrospira platensis* (Ap) is a filamentous helical-shaped cyanobacterium of 200-250 μm in length, with important biotechnological and nutritional applications in the development of foods for humans and animals, production of pharmaceutics, polysaccharides, and cosmetics (Huarachi-Olivera *et al.*, 2015). This microorganism has a high content of proteins, mineral salts, and vitamins (Rojas *et al.*, 2012). Only 10 grams of *Arthrospira platensis* possess a mean nutritional value of 5.27g of protein, 35.2 UI of vitamin A (as β-carotene), 109 μg of vitamin K, 0.05mg of thiamine (vitamin B1), 0.453mg of riboflavin (Vitamin B2), 1.49 mg of niacin (Vitamin B3), 0.096 mg of vitamin B6 (pyridoxine), 16.2 μg of cobalamin (vitamin B12), 0.009-0.992 mg of folic acid (vitamin B9), 0.51 g of tocopherol, and 0.145 g of carotenoids (Finamore *et al.*, 2017). Furthermore, this cyanobacterium is a source of bioactive compounds of therapeutic interest, including calcium-spirulan, immulina, and γ-linolenic acid (GLA) (Furmaniak *et al.*, 2017), polysaccharides, phenols, flavonoids, phycocyanin, sodium-espirulan, chlorophyll, superoxide dismutase (SOD), and zeaxanthin (Sharoba, 2014; Gutiérrez-Salmeán et al., 2015). Moreover, *A. platensis* possesses the GRAS status (Generally Recognized as Safe) by the FDA and is intended for use at levels of 0.5-3 grams per serving in a wide range of food matrixes without adverse effects according to human clinical studies (FDA, 2012).

Although the inclusion of *A. platensis* in pasta has been previously discussed by several studies (Zouari et al., 2011; Lemes et al., 2012; Torres et al., 2014; Hussein et al., 2021; Raczyk et al., 2022), the assessing of a soft wheat pasta formulation added with egg and *A. platensis* on the nutritional value, and the effect of cooking on antioxidant capacity and pigment content of chlorophylls and total carotenoids have not been discussed yet. Given that, this study aimed to measure the effect of adding *Arthrospira platensis* on the nutritional value of pasta, and the effect of cooking on total phenolic content, antioxidant capacity, chlorophylls (a+b), and total carotenoids content.

MATERIALS AND METHODS

Pasta formulation and nutritional value determination

The base formula for the development of pasta was elaborated through the mixing of standard white soft wheat flour (69.83%), water (18.16%), egg yolk (11.31%), and salt (0.70%). Then, the base formula was added with 0 (control), 1, 5, and 10% of dried biomass of *A. platensis* from a commercially available product. The ingredients were mixed and kneaded for 10 min, followed by a rest of 90 min at room temperature. Doughs obtained were processed using an Italian pasta hand press of 15 cm (Vencort, model: 349003), getting tagliatelle-shaped pasta with dimensions of 100x5x1mm. Finally, the pastas were dried for 48 h at room temperature and then used to perform the analysis of proteins (method 991.22; AOAC), fats (method 933.05; AOAC), carbohydrates (by difference), fiber (method 985.29; AOAC), and moisture (method 948.12; AOAC). Caloric Value was calculated by the product of the total content (g) of proteins, fats and carbohydrates using the Atwater system and multiplying each macronutrient content by 4, 9 and 4 kcal respectively.

Uncooked and cooked pasta processing

Ten grams of each pasta were boiled in 200 mL of distilled water for eight min (time necessary for "al dente" term). Then, uncooked and cooked pasta were lyophilized (Labconco, FreeZone 2.5 Liter Benchtop Freeze Dryer model 7670020) for 24h. Finally, pastas were milled into powder using a blender for further analysis.

Preparation of extracts for analytical procedures

One gram of milled pasta was mixed with 9 mL of methanol (80%) into a tube and vortexed for 1 min. Samples were sonicated for 30 min at 30°C (Sonicator VWR model: 150D) and centrifugated (Eppendorf model 5804R) at 7563 g for 5 min at 20°C. Finally, 3 mL aliquots of supernatant were transferred to microtubes and stored in dark conditions at -80°C until their use for antioxidant capacity assays.

Total phenolic content (TPC)

The TPC was determined according to López-Martínez et al. (2022) adapted to microplate. For that, 20 μL of the extract was mixed with 20 μL of Folin-Ciocalteu reagent (Sigma-Aldrich®). After 5 min of incubation, 20 μL of 0.01 M Na_2CO_3 were added and left to react for another 5 min. Finally, 125 μL of distilled water was added and the absorbance was measured at 790 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific). The TPC was calculated from linear regression using a gallic acid solution (0-1500 μmol) and expressed as μmol of gallic acid equivalents per g of dried sample (μmolGAE/gdw).

Antioxidant capacity

ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) assay

The ABTS assay was performed according to Re et al. (1999) adapted to the microplate. To obtain the ABTS free radical (ABTS•+), 5mL of a 7 mM ABTS solution was mixed with 2.5mL of a 2 mM potassium persulfate ($K_2S_2O_8$) solution and incubated for 12 h in the dark at room temperature. After that, absolute ethanol was added to the ABTS•+ mixture until obtaining an absorbance value of 0.70 at 734 nm. For the assay, 10 µL of the sample were placed in a 96-well microplate and mixed with 190 µL of the ABTS•+. After one minute, absorbance was recorded at 734 nm using absolute ethanol as a blank in a microplate reader (Multiskan GO, Thermo Fisher Scientific, Finland). Each sample was analyzed in triplicate. Antioxidant activity was obtained by linear regression using a calibration curve of Trolox (0-800 µmol) and expressed as µmol of Trolox equivalents per gram of sample in dry weight (µmolTE/gdw).

FRAP (Ferric Reducing Antioxidant Capacity) assay

The FRAP assay allows determinate the antioxidant power of a sample based on the reduction at low pH of ferric-tripyridyltriazine (Fe^{+3}-TPTZ) to an intense blue color ferrous-tripyridyltriazine complex (Fe^{+2}-TPTZ). The FRAP assay was developed by adapting the methodology of García et al. (2011) and Alvarez-Parrilla et al. (2005). The FRAP reagent was prepared by mixing 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (Sigma-Aldrich®) in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride. The mixture was incubated for 20 min at 37°C before use. For the assay, 6 µL of extract were placed in a 96-well microplate and mixed with 18 µL of distilled water and 180 µL of FRAP reagent. Methanol (80%) was used as a blank. Absorbance was measured at 593 nm (Multiskan GO, Thermo Fisher Scientific). The antioxidant power was obtained by linear regression using Iron (II) sulfate heptahydrate solutions (0-3000 µmol) and expressed as µmol of Fe^{+2} per gram of dried sample (µmolFe^{+2}/gd*w*).

Pigments determination

Chlorophylls a + b, as well as total carotenoid content, were measured by adapting the technique of Braniša et al. (2013). Briefly, 0.5 g of sample powder was placed in assay tubes with 5ml of acetone (100%), sonicated (3 min), and centrifugated (7563 x g for 10 min, 20°C). The supernatant was stored in amber microtubes of 2 mL at -80°C until its use. For pigments determination, 200 µL of each extract was placed in a 96-well microplate, and absorbance was measured in a microplate reader (Multiskan GO, Thermo Fisher Scientific) at 662nm, 645nm, and 470nm for chlorophyll a, chlorophyll b and total carotenoids, respectively. Obtained values were used in the following equations (Eq. 1-3), according to Lichtenthaler and Wellburn (1983), and then the results were expressed as milligrams/100g of sample dry weight.

$$\text{Chlorophyll a } (\mu g/mL) = 11.75A''662'' - 2.35A''645'' \quad (1)$$

$$\text{Chlorophyll b } (\mu g/mL) = 18.61A''645'' - 3.96A''662'' \quad (2)$$

$$Total\ Carotenoids\ \left(\frac{\mu g}{m\ L}\right) = \frac{1000A470 - 2.27\ (Chla) - 81.4\ (Chlb)}{227} \quad (3)$$

Statistical analysis

All samples were analyzed in triplicate. Statistics analysis were performed using SAS software (SAS 9.0) by one-way ANOVA followed by a Tukey test ($p<0.05$) in the case of nutritional analysis. Regarding antioxidant capacity assays and pigment content estimations, raw and cooked pastas were analyzed separately. A one-way ANOVA followed by a Tukey test ($p<0.05$) was used to measure the differences between the raw pastas, and another one-way ANOVA followed by a Tukey test ($p<0.05$) to measure the differences between the cooked ones.

RESULTS AND DISCUSSION

Effect on nutritional value

The nutritional composition of the formulations elaborated is shown in Table 1.

Ash content shown a tendency of increase in pastas added 1 and 5 % respect control, but not for pasta added at 10%. Other studies (Lemes et al., 2012; Hussein et al, 2021; Muresan et al., 2016) have found increases in ashes due to the content of mineral salts contained on *A. platensis* but the increases vary considerably from 0.08-0.56 to 0.72-1.23 for pastas added at 5 and 10% respectively. In pasta added at 10%, the lack of tendency is probably caused by a partial heterogeneity in the pasta matrix, which affected the mean value of ashes in the proximal analysis. A negative correlation was observed between fat content and the percentage of added *A. platensis*. Contrary to our results, previous studies evidenced fat increases depending on the freshness of the *A. platensis* used. Lemes *et al.* (2012) found increases in fat of 5.9 to 11g using fresh *A. platensis,* while Hussein et al. (2021) reported increases of 0.27 to 0.52g using dried *A. platensis* at 5 and 10% respectively.

Fiber content showed a significant increase ($p<0.05$) in pasta added with *A. platensis* of 22.97, 41.89, and 54.05%, for 1, 5, and 10% respectively. These results are

Table 1: Nutritional value per 100 grams (wet weight) of pasta added with *A. platensis*

Component/Pasta	0%	1%	5%	10%
Moisture (%)	9.65±0.05^{c}	10.98±0.02^{a}	8.34±0.05^{d}	9.87±0.15^{b}
Ash (g)	0.95±0.10^{a}	1.08±0.06^{a}	1.1±0.01^{a}	0.86±0.21^{a}
Crude Fiber (g)	0.74±0.02^{b}	0.91±0.19ba	1.05±0.01^{a}	1.14±0.04^{a}
Fat (g)	4.59±0.02^{a}	4.31±0.02^{b}	3.69±0.09^{c}	3.57±0.14^{c}
Protein (g)	12.45±0.30^{b}	13.22±0.10^{b}	14.85±0.4^{a}	15.87±0.63^{a}
Assimilable Carbs (g)	71.62±0.38^{a}	69.5±0.10^{b}	70.97±0.36^{a}	68.69±0.63^{b}
Energy (Kcal)	377.59±0.17^{a}	369.67±0.63^{b}	376.49±0.66^{a}	370.37±1.1^{b}

Values are means (n=3) ± SD. Different lowercase letters denote a significant difference (P<0.05).

in agreement with previously reported values ranging from 2.29-40.38% to 38.80-76.92% for pastas added at 5 and 10% respectively (Lemes et al., 2012; Hussein et al, 2021). Moisture, carbohydrates, and energy parameters showed statistically significant differences between the formulations, but it was not detected a tendency related to adding *A. platensis*.

There is scarce information about centesimals analyses on moisture content of *A. platensis* added pasta but some insights can be found in the literature. Lemes et al. (2012) when measuring the moisture content of pastas added at 5 and 10% with *A. platensis* found no significative differences between the added pastas and control. The authors stated that the differences found in the centesimal analysis could be attributed to a lack of homogenization during pasta processing, analytical inaccuracies, as ingredient of the same lot were used. Nonetheless, their results were performed on fresh pastas. In contrast, Muresan et al. (2016) performed centesimal analyses on *A. platensis* added pastas and found increased moisture content of 7.21, 7.37, and 7.59 in function of the *A. platensis* added for control, 2% and 5% *A. platensis* added pasta respectively. The difference in moisture content shown here could be attributed to the non-homogeneous dehydration of pasta due to a partial homogenization during pasta making process.

Regarding control, the formulations added at 5 and 10% showed significant increases ($P < 0.05$) in protein content of 19.27 and 27.46%, respectively. Other authors have found increases in the protein content of pasta supplemented with *A. platensis* of 7-27 and 35-54% (Lemes et al., 2012; Hussein et al., 2021) for pasta added at 5 and 10%, respectively. However, the overall differences between the changes produced in the nutrimental profile regarding protein, carbohydrates, fats, fiber, and ashes content provided by each percentage of addition of *A. platensis* found here and those described in the literature are possibly due to the high susceptibility of the *A. platensis* nutritional profile to variables such as strain used, its growth media, and its freshness (Lemes *et al.*, 2012).

The *A. platensis* used in this work enhanced the protein content of pasta added at 5 and 10%. The FDA categorizes foods with a 20% DV of protein per portion as high protein foods. The %DV of protein provided per portion of dry *pasta added* at 5 and 10% with *A. platensis* is equivalent to 17 and 18% respectively. Hence, their protein content is close to the category of high-protein foods established by the FDA.

Total phenolic content (TPC) of pasta

Phenolics are an important group of compounds that act as primary antioxidants or free radical terminators (Papalia *et al.*, 2017), and the consumption of foods rich in them has been linked to a lowered risk of chronic and many degenerative diseases (Tsao, 2010; Gutiérrez-Grijalva et al., 2016). Studies indicate that phenolics, carotenoids, chlorophylls (and their degradation products such as pheophytins), and phycocyanin are responsible for the antioxidant properties of *A. platensis* (Jaime *et al.*, 2005; Park *et al.*, 2018). The results of the TPC and the antioxidant capacity by ABTS and FRAP assays are shown in Table 2.

The Total phenolic content of uncooked and cooked control pasta (0%) was 43.79 y 38.07 mg GAE/g respectively. Regarding to the others formulations, an increase in the total phenolic content was shown proportional to the addition of *A. platensis*. Pastas added at 1, 5 and 10% of Ap increased their TPC by 44.49, 45.49 y 46.35 mg GAE/g and 38.67, 42.12 y 45.41 mg GAE/g for uncooked and cooked pastas respectively.

Statistically, the differences between control pasta, and uncooked and cooked pasta with 10% of Ap were significant, reaching values of 2.56 and 7.34 mgGAE/g respectively, higher than the control. The increases produced in uncooked pasta by *A. platensis* addition at 5 and 10% were 1.7 and 2.56 mgGAE/g respectively. These results are similar to those previously discussed by Hussein *et al.* (2021) who also fabricated pastas added with *A. platensis* at 5 and 10% and found increases of 1.31 and 2.27 mg/g respectively in uncooked pastas. Nonetheless in the literature, the phenolic increases produced by *A.* platensis in pasta vary to a great extent from values of 0.5mg/g (Koli *et al.*, 2022) to 3mg/g (Hussein *et al.*, 2021). However, it is worth mentioning that the growth media influence to a great extent the phenolic composition

Table 2: TPC, ABTS, and FRAP results of pasta added with *A. platensis*

PASTA AP addition (%)	Uncooked		Cooked		Method
	(mg GAE/g)	Increase (%)	(mg GAE/g)	Increase (%)	
0	43.79±0.40^{c}		38.07±0.61^{c}		TPC
1	44.49±0.51^{bc}	1.59	38.67±0.24^{c}	1.57	
5	45.49±0.67^{ba}	3.88	42.12±0.40^{b}	10.63	
10	46.35±0.59^{a}	5.84	45.41±0.06^{a}	19.28	
	(μmolTE/g)	Increase (%)	(μmolTE/g)	Increase (%)	
0	9.29±0.39^{c}		7.07±0.51^{c}		ABTS
1	10.7±0.97^{c}	15.17	7.3±0.6^{c}	3.25	
5	13.8±0.95^{b}	48.54	11.48±041^{b}	62.37	
10	16.8±0.13^{a}	80.83	14.62±0.11^{a}	106.78	
	(μmolFe^{+2}/g)	Increase (%)	(μmolFe^{+2}/g)	Increase (%)	
0	11.65±0.18^{d}		11.28±0.59^{d}		FRAP
1	13.3±0.35^{c}	14.16	12.8±0.52^{c}	13.47	
5	19.35±0. 47^{a}	66.09	19.25±0.08^{b}	70.65	
10	18.13±0.17^{b}	55.62	21.42±0.41^{a}	89.89	

Values are means (n=3) ± SD. Different lowercase letters denote a significant difference (P<0.05).

of microalgae (Safafar et al., 2015; Guldas et al., 2020). The increase shown in the TPC assay performed here can be attributed to the content of *A. platensis* in gallic, caffeic p-Hydroxybenzoic, p-cumaric, ferulic, vanillic, syringic, chlorogenic, protocatechuic acids, and catechin, quercetin, genistein, kaempferol, 4-hydroxybenzaldehyde, 3,4-dihidroxybenzaldehyde, (Klejdus *et al.*, 2009; Papalia *et al.*, 2017).

Antioxidant capacity

Antioxidants are important molecules that play a role in protecting the body against oxidative damage, hence protecting from cardiovascular, carcinogenic, neurological diseases and delaying chronic health problems (Dolas and Gotmare, 2015). An antioxidant is defined as a substance that, decreases or prevents in a significant way the adverse effects of reactive species (free radicals) of oxygen and nitrogen on normal physiological function in humans (Karadag et al., 2009). *A. platensis* is a source of bioactive compounds, especially antioxidants such as phenolics, protein complexes, antioxidant enzymes, polyunsaturated fatty acids, protein-pigment complexes (phycobiliproteins), and pigments like chlorophylls, xanthophylls, and carotenoids that can be used in the development of foods, pharmacological and biotechnological products (Klejdus et al., 2009; Finamore et al., 2017; Sannasimuthu et al., 2018).

ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) assay of Pasta

The free radical scavenging capacity by ABTS of pasta is shown in Table 2. Since the DPPH assay have been used commonly to describe the antioxidant capacity of pastas added with *Arthrospira platensis* (Zouari et al., 2011; Muresan et al., 2016; Hussein et al., 2021; Koli et al., 2022), a DPPH assay was carried out additionally to the ABTS performed here; however, it was not included in the document since the results were not concise, and there was not a tendency related to the adding of *A. platensis*. Instead, the ABTS assay showed better results in contrast to those obtained by the DPPH assay. This non-sensitivity towards the antioxidant capacity of pastas added with *A. platensis* showed by the DPPH is consistent with the described in the study of Matos et al. (2020) in which the antioxidant activity of aqueous and ethanolic extracts of *A. platensis* was measured by DPPH, FRAP and ABTS. The authors found that both *A. platensis* aqueous and ethanolic extracts showed not results for antioxidant activity by DPPH, but for FRAP and ABTS. Furthermore, the authors stated that FRAP, ABTS and especially oxygen radical absorbance capacity (ORAC) assays could be more valuable than DPPH. However, at the moment there is no information about previous ABTS assays performed on formulations as the presented here to compare for reference values. Nonetheless, the results shown in this work could be helpful for further analyses on similar pasta formulations by this method.

All uncooked formulations added with *A. platensis* increased their antioxidant capacity by 15.17%, 48.54%, and 80.83% for samples added at 1, 5, and 10%, respectively, in contrast to the control (9.29 μmolTE/g). After the cooking process, samples decreased their antioxidant capacity concerning uncooked samples by 32, 17, and 13% for 1, 5, and 10%, respectively. However, they remained higher by 3.25, 62.37, and 106.78% for 1, 5, and 10% respectively than cooked control (7.07 μmolTE/g). Nonetheless, samples added with 5 and 10% increased significantly (P<0.05) their antioxidant capacity evidenced by Trolox equivalents.

Ferric reducing antioxidant power (FRAP) of pasta

The FRAP assay of pastas is shown in Table 2. The uncooked samples added with *A. platensis* showed increases statistically significant (p<0.05) of 14.16, 66.09, and 55.62%

for 1, 5, and 10% samples respectively, in contrast to the control (11.65 μmolFe^{+2}/g). The uncooked pasta added at 5% *A. platensis* showed the highest value in the FRAP assay than uncooked 10% pasta. However, when comparing both samples after the cooking process, the cooked pasta added at 10% with *A. platensis* showed an increase in its ferric reducing capacity (89.89%) in contrast to the 5% cooked sample (70.65%). Nonetheless, both samples remained high in contrast to cooked control pasta (11.28 μmolFe^{+2}/g).

The differences found in the ferric reducing capacity of uncooked 5% and 10% samples and their change when they were cooked could be due to some components present in *A. platensis* that acidify the reaction media of FRAP assay. Ou et al. (2002) when assessed freeze-dried vegetable samples' antioxidant capacity by FRAP, found that the assay presented some drawbacks, one of them related to the pH nature of the assay since the antioxidant must be able to reduce the Ferric complex (Fe^{+3}) to a Ferrous (Fe^{+2}) complex in a pH of 3.6. They suggest that a low pHcan inhibit one-electron transfer from the antioxidant to the ferric ion. In this study, the 10% uncooked pasta has a lower pH than 5% pasta (data not shown) due to the presence of more acidic molecules contained in *A. platensis*. Thus, reducing capacity results appear to be lower in the 10% uncooked pasta possibly by the pH interference previously discussed. After the cooking process, pasta rehydrates, possibly degrades and loses some of its compounds in the cooking media, the pH of pasta is slightly increased, and hence reducing the pH interference of the 10% sample, producing a higher value than the obtained by 5% cooked sample.

Nonetheless, the ferric reducing capacity of 1 and 5% *A. platensis* added cooked pastas showed slight decreases concerning uncooked samples of 4%, and 0.5%, respectively, while 10% pasta showed an increase of 18%. The control sample showed a ferric reducing capacity reduction of 4%. Our results in the FRAP values are in the range of those found in recently published studies (Hussein et al., 2021; Koli et al., 2022).

Estimation of chlorophylls a + b, and total carotenoids in pasta

The colors displayed in fruits and vegetables are provided by the occurrence of three natural families of pigments: chlorophylls (green), carotenoids (red-yellow), and anthocyanins (blue-violet) (Bakan et al., 2014). Since these pigments cannot be synthesized by animals, they rely on their diet to incorporate them from natural sources such as plants, algae, eggs, and fish (Pérez-Gálvez et al., 2020). The use of *A. platensis* as a natural source of pigments such as chlorophylls, carotenoids, and C-phycocyanin (blue pigment) is increasingly more frequent due to the consumer awareness of the importance of these pigments regarding nutritional, pharmacological, and health benefits (Park et al., 2018).

According to Mishra et al. (2011), chlorophyll and derivates (such as pheophytins) possess potent antioxidant and radioprotective effects *in vivo* and *in vitro*, inhibit lipid peroxidation, protein oxidation, and prevent DNA as well as membrane damage. On the other hand, carotenoids are important pigments related to immunity functions, antioxidants, and lowering the risk of developing chronic diseases (Viera et al., 2018).

Since *Arthrospira platensis* is a source of lipophilic green and red pigments (chlorophylls and carotenoids) as well as hydrophilic intense-blue pigments (C-Phycocyanin) (Park et al., 2018), all samples added with *A. platensis* showed a change in their color, attributable to the content in these pigments. The pastas developed in this work are shown in Fig.1.

The content of chlorophylls a + b, and total carotenoids are shown in Table 3.

A positive correlation was observed between chlorophyll a + b and total carotenoids concerning the *A. platensis*

Fig 1. Soft wheat pastas with egg. a) control pasta, b) 1%, c) 5% and d) 10% A. platensis.

Table 3: Chlorophyll (a+b) and total carotenoids content of pasta added with *A. platensis*

PASTA AP addition (%)	Uncooked	Cooked	Determination (mg/100g)
1	1.04±0.06^{c}	2.71±0.01^{c}	
5	5.89±0.25^{b}	11.32±0.32^{b}	Chl (a+b)
10	16.20±0.43^{a}	19.32±0.07^{a}	
1	0.22±0.01^{c}	0.34±0.01^{c}	
5	1.31±0.11^{b}	2.03±0.06^{b}	Total Carotenoids
10	3.72±0.13^{a}	3.39±0.02^{a}	

Values are means (n=3) ± SD. Different lowercase letters denote a significant difference (P<0.05).

addition. The chlorophyll and total carotenoids values obtained by 5 and 10% cooked pasta is 11.32, 19.32 and 2.03, 3,39 mg/100g, respectively. These values are similar to those previously discussed by several studies: Torres et al. (2014) who developed pasta with wheat semolina flour added with 5% *A. platensis* obtained chlorophyll values of 10.74mg/100g of cooked pasta. Igual et al. (2022) showed that the addition of *A. platensis* (1.5%/100g) in breadsticks caused the occurrence of chlorophyll a and carotenoids of approximately 8 mg and 1.75 mg/100g respectively after backing. Furthermore, Tańska et al. (2017) by using extrusion-cooking process fabricated extrudates made of corn grits added with several percentages of *A. platensis*, among them 6 and 8%/100g. The authors found values of chlorophyll a of 12.6, and 20.8 mg/100g respectively.

The content of chlorophyll could be of relevance since previous studies have shown that these pigments possess several biological properties such as the prevention of cancer and other digestive diseases (Delgado-Pelayo *et al.*, 2014). Although the cooking process and digestion of pasta transform all chlorophyll content into chlorophyll derivates such as pheophytins, these derivates continue exerting antioxidant properties, even higher antioxidant activity than the original molecule of chlorophyll a or b according to Hsu *et al.*, 2013.

Since the pasta cooking procedure used in this work did not involve temperatures higher than 100°C for 8 minutes, the pasta cooking process is inside of the suggested safest set indicated by Parwani and Singh (2019) to conserve the good nutritional value, antioxidant properties, and pigment content of *A. platensis*.

The increase in chlorophyll a + b and total carotenoids content in cooked pasta is probably attributed to the structural damage of *A. platensis* induced by the cooking process, which also caused a weakened gluten network by the swelling of the starch granules damaging the network of the pasta (Bruneel et al., 2010). Furthermore, experimentally, cooked pasta was more brittle and permeable than uncooked pasta to the extraction media, hence it was expected more interaction between solvents used during preparation of extracts and cooked pastas.

The results in pasta developed in this work evidenced limited bleaching and an increase in the content of carotenoids and chlorophylls. Britton and Khachik (2009) suggested a helpful categorization criterion for carotenoid content in foods, with four levels; low (0 - 0.1mg/100g), moderate (0.1 - 0.5mg/100 g) high: (0.5 – 2 mg/100g) and very high (>2mg/100g). Pasta formulations added at 5 and 10% with *A. platensis* can be classified as high in carotenoids, representing an option to complement the daily ingest of these pigments. According to Meléndez-Martínez et al. (2021) an optimal carotenoid intake may be related to the reduction of risk of developing certain cancers (cervical, ovarian, colorectal, prostate, breast), cardiovascular disease, bone, skin, or eye disorders.

CONCLUSIONS

The addition of *A. platensis* at 5% was the minimum necessary to produce a pasta rich in proteins (19.27%), fiber (41.89%), and increased antioxidant capacity (more than 48%) as well as pigments such as chlorophylls (5.89 mg/100g) and carotenoids (1.31mg/100g) in raw pasta without statistically significant modifications in the caloric value. After the cooking process, pasta added at 5% decreased its antioxidant capacity in 7.40, 16.81 and 0.51% for TPC, ABTS and FRAP respectively, however the resultant values remained 10.63, 62.37 and 70.65 higher than cooked control. Furthermore, increases of 92.19 and 54.46% were noticed for chlorophyll (a+b) and total carotenoids respectively. The addition of *A. platensis* represents a simple and feasible way to increase the content of proteins and bioactive compounds, using a common wheat refined flour of affordable price. This study showed that the addition of *A. platensis* in a non-previously described formulation comprised by soft wheat flour and egg is able to produce pasta with potential health benefits by enriched nutrimental value regarding proteins, increased phenolic content, enhanced antioxidant capacity, and abundant in pigments such as chlorop hylls and carotenoids. Further studies assessing the antioxidant capacity of whole wheat pasta formulations added with egg and *A. platensis* could be of interest since in addition to its antioxidant properties it can contribute to the daily ingest of fibers. On other hand, other grain pasta alternatives such as lentil or chickpea pasta added with *A. platensis* represent a way to distribute the bioactive compounds through a high protein and fiber matrix to the allergen reactive population or those with special diet requirements.

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Authors' Contributions

García-Moncayo, A. I: Conducted the research work and manuscript writing; Rodríguez-Martínez, E. S: Analytical assistance in nutritional determination data; Ochoa-Reyes, E.: Analytical assistance in antioxidant assays; Sáenz-Hidalgo, H. K: Nutritional value determination of pasta samples; Sepúlveda, Ahumada, D. R.: Guidance

and evaluation of the manuscript; Buenrostro-Figueroa, J. J: Guidance and correction of the manuscript; Alvarado-González, Monica: Guidance, research structure, and correction of the manuscript.

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